Biochemical Pharmacology, Vol. 36, No. 3, pp. 369–375, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

STIMULATION OF PROSTAGLANDIN PRODUCTION BY (2E,6Z,10E)-7-HYDROXYMETHYL-3,11,15-TRIMETHYL-2,6,10,14-HEXADECATETRAEN-1-OL (PLAUNOTOL), A NEW ANTI-ULCER DRUG, *IN VITRO* AND *IN VIVO*

SHIGERU USHIYAMA,* KEIICHI MATSUDA, FUMITOSHI ASAI and MITSUO YAMAZAKI
Biological Research Laboratories, Sankyo Co., Ltd., Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(*Received* 3 *April* 1986; *accepted* 10 *July* 1986)

Abstract—Plaunotol [(2E,6Z,10E)-7-hydroxymethyl-3,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol], a new anti-ulcer drug, was investigated for its effect on prostaglandin (PG) production. In cultured cells of 3T6 fibroblasts, plaunotol and its main metabolite (1-carboxylic plaunotol) at concentrations of 10–100 μM increased PGE_2 and PGI_2 production 2- to 4-fold. These compounds increased the release of radioactive arachidonic acid from [^{14}C]arachidonic acid prelabeled 3T6 fibroblast cells 2-fold at 30 μM, and this increase was inhibited by addition of mepacrine, a phospholipase inhibitor. Plaunotol and its main metabolite had no effect on PG cyclooxygenase activity. These results indicate that plaunotol and its main metabolite stimulate PG production by activating cellular phospholipase. In gastric-mucosa slices, PGE_2 and PGI_2 production was increased significantly either by oral administration of plaunotol to rats at a dose of 300 mg/kg or by addition of the main metabolite to the incubation medium. All these results suggest that plaunotol increases the PG levels in gastric mucosa by stimulating the PG biosynthesis, particularly the cellular phospholipase activity. The increased levels of PG may participate in the anti-ulcer activity of plaunotol.

Plaunotol is a new acyclic diterpene alcohol extracted from a Thai medicinal plant, plau-noi, *Croton sublyratus* Kurz [1]. It has shown excellent anti-ulcer activity both in experimental ulcers induced by reserpine, stress, aspirin and indomethacin [2, 3] and in clinical trials [4]. Plaunotol increases the gastric-mucosa blood flow [5], enhances the hexosamine content in the mucosa and increases the uptake of [^{3}H]-D-glucose by the gastric tissue in rats [6].

Prostaglandin E_2 and I_2 (PGE_2 and PGI_2) have been shown to inhibit gastric acid secretion [7–11], enhance local blood flow [12, 13], and stimulate mucus secretion [14] in the stomach in experimental animals and humans. These PGs exert a cytoprotective effect on gastric mucosa injured by a variety of necrotizing agents [15, 16], and they prevent ulcer formation in animals [8, 17, 18] and promote ulcer healing in humans [19, 20]. Some of the actions of plaunotol on the stomach are very similar to those of PGs.

The present study was undertaken to elucidate how PGs contribute to the pharmacological activity of plaunotol. To examine the stimulatory activity of plaunotol on PG production at the cellular level, an established cell line of 3T6 fibroblasts was used, because fibroblasts produce large amounts of PGs in the medium [21]. We found that plaunotol and its main metabolite markedly enhanced PG production through activation of cellular phospholipase in 3T6 cells. The effect of plaunotol on PG production was further examined in rat gastric mucosa *in vitro* and *ex vivo*. The results of our study suggest that plaunotol exerts its activity through the elevation of endogeneous PG levels in gastric mucosa.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Plaunotol and its metabolites were prepared by the Sankyo Co., Ltd. (Tokyo). [1-^{14}C]Arachidonic acid and the radioimmunoassay kit for 6-keto-$PGF_{1\alpha}$ were purchased from the New England Nuclear Corp. (Boston, MA); the radioimmunoassay kit for PGE was from Clinical Assays (Cambridge, MA) and [^{14}C]PGE_2 from Amersham International (Buckinghamshire, U.K.). Gefarnate and cetraxate were products of Teikoku Chemical Industry (Osaka) and the Daiichi Pharmaceutical Co., Ltd. (Tokyo) respectively. Dulbecco's modified Eagle Medium (Dulbecco-MEM) was obtained from the Nissui Pharmaceutical Co., Ltd. (Tokyo). 3T6 Fibroblast cells, a cultured strain from Swiss albino mice, were obtained from the Tissue Culture Center of the Dainippon Pharmaceutical Co., Ltd. (Osaka). In the *in vitro* experiments, compounds were dissolved in ethanol (final ethanol concentration was less than 0.1%). In the *ex vivo* experiments, plaunotol was administered orally in the form of an emulsion with Tween-80 (0.4%).

Estimation of PGE_2 and PGI_2 production in 3T6 fibroblast cells. The effects of plaunotol and its metabolites on PGE_2 and PGI_2 production in 3T6 fibroblast cells were examined according to the previously reported method [21], with a modification. 3T6 Fibroblast cells (2×10^5 cells/well) were seeded and cultured in 2 ml of Dulbecco-MEM supplemented with 10% fetal calf serum (FCS) in a multi-well plate (12 wells, 24 mm in diameter; Terumo Co., Tokyo) at 37° in an atmosphere of 95% air and 5% CO_2. After 48 hr of cultivation, the cells (8×10^5 cells/well) were washed twice with 2 ml of serum-free medium and were incubated in 1 ml of

fresh medium supplemented with 10% FCS containing plaunotol or one of the other compounds to be tested. After a 3-hr incubation, the medium was collected and radioimmunoassayed for PGE_2 and 6-keto-$PGF_{1\alpha}$. PGE_2 was converted to PGB_2 by heating at 80° for 10 min in 0.1 N NaOH and was assayed using rabbit anti-PGB serum. PGI_2 was assayed as 6-keto-$PGF_{1\alpha}$ using rabbit anti-6-keto-$PGF_{1\alpha}$ serum.

Cell viability was determined by staining with trypan blue.

Arachidonic acid release from the prelabeled 3T6 fibroblast cells. The effects of plaunotol and its metabolites on arachidonic acid release were examined using [^{14}C]arachidonic acid prelabeled 3T6 fibroblast cells according to the method of Hong and Levine [22]. 3T6 Cells (5×10^5 cells/well) were incubated with [^{14}C]arachidonic acid (0.1 μCi/ml; specific activity 52 mCi/mmole) in 2 ml of Dulbecco-MEM supplemented with 10% FCS at 37° for 24 hr. The prelabeled cells were then washed twice with 2 ml of serum-free medium and were incubated in 1 ml of fresh medium supplemented with 10% FCS containing plaunotol or one of the other compounds to be tested. After a 3-hr incubation, the radioactivity released into the medium was counted in a liquid scintillation counter. The radioactive substances in the medium were extracted with 5 vol. of ethyl acetate after acidification of the medium with 10% formic acid to pH 3.0. The ethyl acetate layer was dried under an N_2 gas stream, and the residue was dissolved in a small amount of methanol and applied to a thin-layer plate (Merck, Kieselgel 60F). The developing solvent was an organic phase of ethyl acetate, acetic acid, iso-octane, and water (11:2:5:10). The radioactive substances were detected with a radioactive scanner (Berthold Co.).

Cellular phospholipids were extracted and separated by the method of Bligh and Dyer [23], and analyzed by thin-layer chromatography according to the method of Wagner and Horhammer [24].

Assay of PG cyclooxygenase activity. The effects of plaunotol and its metabolites on PG cyclooxygenase were examined by the previously reported method [25] with a slight modification using the microsomes of bovine seminal vesicle and 3T6 fibroblast cells. A microsomal fraction of bovine seminal vesicles was prepared according to the method of Takeguchi *et al.* [26]. The microsomes of 3T6 fibroblast cells were prepared by the same method after the cells (1.5×10^8 cells) were sonicated by a Branson sonicator twice for 30 sec. The reaction mixture contained 100 mM Tris–HCl buffer (pH 7.4), 20 μg/ml

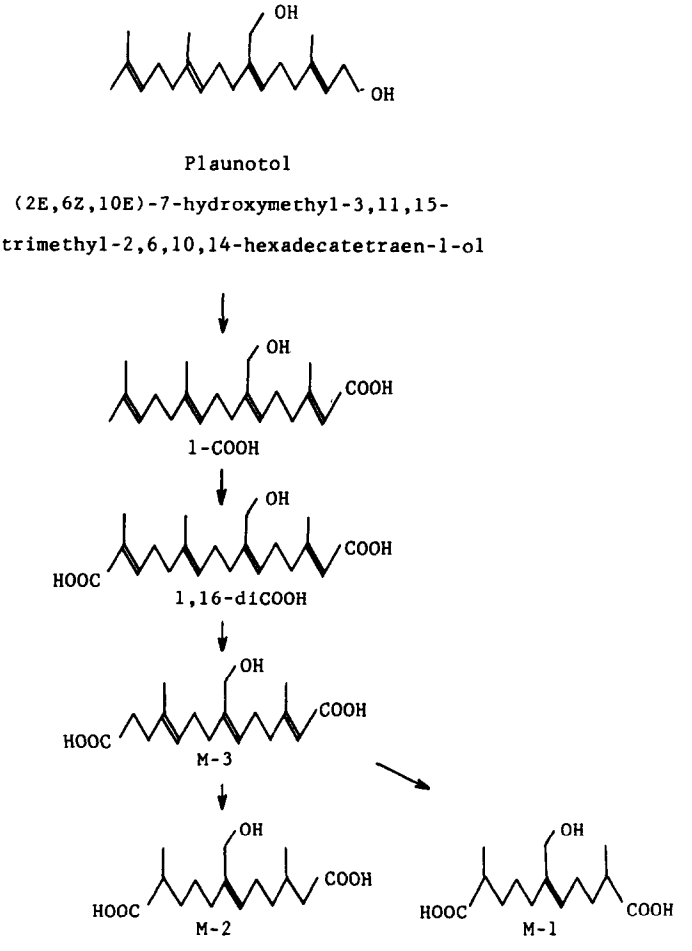

Fig. 1. Structures of plaunotol and its metabolites. The metabolic pathway of plaunotol is shown by arrows. 1-COOH (1-carboxylic plaunotol) is the main metabolite in plasma.

of hemoglobin, 2 mM glutathione, 400 μg of the microsomal enzyme, and the compound to be tested (dissolved in 4 μl ethanol), in a total volume of 200 μl. The reaction was started by adding 3.2 nmoles of [^{14}C]arachidonic acid (0.17 μCi) dissolved in 5 μl ethanol and terminated by adding 50 μl of 0.2 N citric acid after a 10-min incubation at 30°. The PGE_2 formed was extracted with 1.5 ml of ethyl acetate and determined by silica gel thin-layer chromatography.

Estimation of PGE_2 and PGI_2 production in gastric-mucosa slices in vitro. Male rats (Donryu strain) weighing approximately 250 g were killed, the stomach was taken out, and the glandular mucosa was dissected into slices (2 mm width) in ice-cold Krebs–Ringer bicarbonate solution (120 mM NaCl, 4.8 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 26 mM $NaHCO_3$ and 10 mM glucose), pH 7.4. PG production from the mucosal slices was estimated according to the method of Spławińska *et al.* [27]. The slices (approximately 400 mg) from one rat were divided into two parts. Each part was vortexed vigorously for 30 sec, rinsed twice, and then incubated in 10 ml of Krebs–Ringer solution with or without plaunotol or its 1-COOH metabolite at 37°. Aliquots of the incubation medium were withdrawn at the appropriate intervals, and PGE_2 and 6-keto-$PGF_{1\alpha}$ released into the medium were determined by radioimmunoassay.

Estimation of gastric-mucosa PG production in the plaunotol-administered rat. Male rats were starved for 20 hr and divided into two groups. The experimental group was given plaunotol orally, while the control group was given the vehicle: the rats were killed 90 min later. The stomach was taken out, and slices of the mucosa (2 mm width) were prepared in ice-cold Krebs–Ringer solution. Approximately 300 mg of the slices was incubated in 10 ml of fresh Krebs–Ringer solution aerated with 95% O_2–5% CO_2 at 37°. Aliquots of the medium were withdrawn at intervals, and the levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the medium were radioimmunoassayed.

The effect of plaunotol on gastric-mucosa PG production was also investigated in aspirin-treated rats. The rats were given plaunotol orally 60 min before aspirin administration and were killed 15 min after aspirin administration; subsequent procedures were as described above.

RESULTS

Increase in PGE_2 and PGI_2 production in 3T6 fibroblast cells. Plaunotol and its metabolites (Fig. 1) were tested for their effects on PGE_2 and PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) production from 3T6 fibroblast cells. Plaunotol and its 1-COOH metabolite, the main metabolite of plaunotol in plasma, stimulated PG production in a concentration-dependent manner and showed approximately 2-fold elevation at a concentration of 30 μM (Table 1). The 1-COOH metabolite was a more potent stimulator than plaunotol. The other metabolites (1,16-diCOOH, M-3, M-2, and M-1) and other anti-ulcer drugs, gefarnate and cetraxate, had no effect on PG production (Table 1).

Activation of cellular phospholipase in 3T6 cells. Plaunotol and its metabolites were examined for their stimulatory effects on cellular phospholipase. When the 3T6 cells were incubated with [^{14}C]arachidonic acid at 37° for 24 hr, about 60% of the radioactivity was incorporated into the cells, and more than 90% of the radioactivity incorporated was associated with phospholipids. The prelabeled cells were incubated with plaunotol or its metabolites for 3 hr, and the radioactivity that was released into the medium was determined. The release of radioactivity was increased dose dependently by plaunotol and its 1-COOH metabolite (1.9- and 2.2-fold at 30 μM respectively) but not by other metabolites of plaunotol and other anti-ulcer drugs, gefarnate and cetraxate (Table 2). When mepacrine, a phospholipase inhibitor, was preincubated with the cells at a concentration of 100 μM, the release of radioactivity decreased about 50–60% both in the control and in the plaunotol or its 1-COOH metabolite-treated cells (Table 2).

The radioactive substances in the medium were analyzed by thin-layer chromatography. As shown in Fig. 2, two major peaks appeared in the chromatogram: the higher peak was arachidonic acid (about 65% of the released radioactivity) and the other peak was PGE_2 (about 20%). Plaunotol stimulated this release of radioactive arachidonic acid and the subsequent production of radioactive PGE_2 about 2-fold at 30 μM (Fig. 2). The 1-COOH metabolite showed the same stimulatory activity.

Effect on PG cyclooxygenase activity. Plaunotol and its metabolites were examined for their effects

Table 1. Increase in PG production by plaunotol and its 1-COOH metabolite in 3T6 fibroblast cells

	Concn (μM)	PGE_2 released (ng/10^6 cells)	PGI_2 released (ng/10^6 cells)
Expt. 1			
None		4.8 ± 0.8	0.67 ± 0.23
Plaunotol	10	6.4 ± 1.1	0.87 ± 0.22
	30	8.3 ± 1.5	1.4 ± 0.4
	100	8.3 ± 1.1	1.7 ± 0.7
1-COOH	10	9.2 ± 1.6	1.2 ± 0.4
	30	11.4 ± 1.7	1.6 ± 0.6
	100	14.2 ± 2.1	2.5 ± 0.8
Expt. 2			
None		5.6 ± 0.4	0.34 ± 0.03
Plaunotol	100	10.6 ± 1.0	1.28 ± 0.11
1-COOH	100	10.6 ± 0.8	1.34 ± 0.05
1,16-diCOOH	100	5.8 ± 0.1	0.48 ± 0.05
M-3	100	5.8 ± 0.6	0.42 ± 0.01
M-2	100	6.6 ± 0.1	0.57 ± 0.10
M-1	100	5.5 ± 0.3	0.44 ± 0.07
Expt. 3			
None		2.4 ± 0.1	1.1 ± 0.09
Gefarnate	100	2.1 ± 0.2	1.3 ± 0.19
Cetraxate	100	2.0 ± 0.2	1.0 ± 0.07

3T6 Cells (8×10^5 cells/well) were incubated with the compound for 3 hr in Dulbecco-MEM supplemented with 10% FCS. The amounts of PGE_2 and PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) released into the medium during the incubation were determined by radioimmunoassay as described in Materials and Methods. The viability of the cells was more than 95%. Each value is the means ± SEM of three determinations.

Table 2. Stimulation of the release of radioactivity from prelabeled 3T6 fibroblast cells by plaunotol and its 1-COOH metabolite

	Radioactivity released (dpm/10^6 cells)
Expt. 1	
None	4357 ± 254
Plaunotol (10 μM)	6109 ± 56
(30 μM)	8089 ± 447
1-COOH (10 μM)	7184 ± 933
(30 μM)	9671 ± 198
1,16-diCOOH (100 μM)	3263 ± 123
M-3 (100 μM)	3329 ± 320
M-2 (100 μM)	3153 ± 49
M-1 (100 μM)	3164 ± 190
Gefarnate (100 μM)	4141 ± 475
Cetraxate (100 μM)	4871 ± 317
Expt. 2	
None	4564 ± 313
Mepacrine (100 μM)	2478 ± 402
Plaunotol (30 μM)	8795 ± 728
Plaunotol (30 μM) + mepacrine (100 μM)	3205 ± 75
1-COOH (30 μM)	9675 ± 835
1-COOH (30 μM) + mepacrine (100 μM)	3539 ± 89

3T6 Cells (5×10^5 cells/well) prelabeled with [^{14}C]arachidonic acid were incubated with the compound for 3 hr. Mepacrine (a phospholipase inhibitor) was preincubated with the cells for 1 hr. After the incubation, the medium was withdrawn and counted for radioactivity as described in Materials and Methods. The viability of the cells was more than 95%. Each value is the mean ± SEM of three determinations.

on PG cyclooxygenase. The cyclooxygenase activity was determined by formation of PGE_2 from [^{14}C]arachidonic acid using the microsomes of bovine seminal vesicle and 3T6 fibroblast cells as the enzyme. These compounds had essentially no effects on the activities of these cyclooxygenases at concentrations as high as 300 μM.

Increase in PG production in rat gastric mucosa in vitro. The stimulatory activities of plaunotol and its main metabolite on gastric-mucosa PG production were investigated *in vitro*. The stomach was removed from a rat and the gastric mucosa was dissected into slices and incubated with the compound in a reaction medium. The amounts of PGE_2 and PGI_2 released into the medium were determined. Figure 3 shows the production of PGE_2 and PGI_2 from the gastric-mucosa slices in the presence or absence of the 1-COOH metabolite at a concentration of 100 μM. Production of PGE_2 was stimulated more markedly than that of PGI_2. The stimulatory effect of plaunotol on PG production was less than in the case of the 1-COOH metabolite.

Stimulation of gastric-mucosa PG production by administration of plaunotol. Plaunotol was given orally to rats at a dose of 300 mg/kg and, after 90 min, gastric mucosa was dissected into slices. The slices were incubated in Krebs–Ringer solution, and the amounts of PGE_2 and PGI_2 in the medium were determined at intervals. In the plaunotol-administered group, the amounts of PGE_2 and PGI_2 released were obviously higher than those in the control group (Fig. 4).

The effect of plaunotol was further investigated in

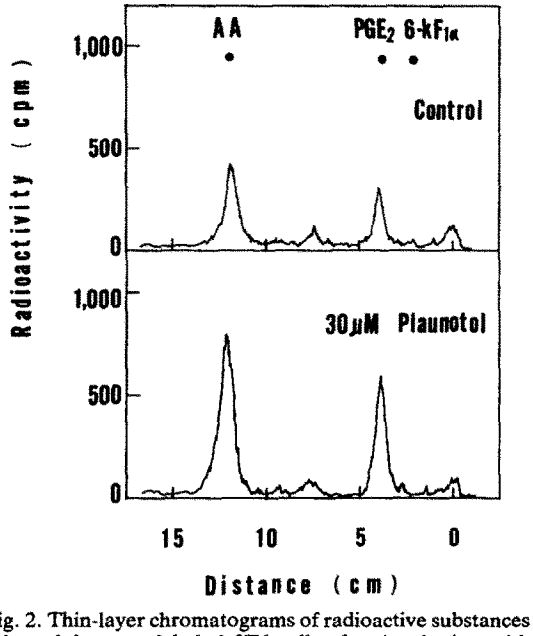

Fig. 2. Thin-layer chromatograms of radioactive substances released from prelabeled 3T6 cells after incubation with plaunotol. The medium (1.6 ml) from two wells (7.5×10^5 cells/ml) was extracted with ethyl acetate. The ethyl acetate extract was applied to a silica gel thin-layer plate. The radioactive substances were detected as described in Materials and Methods. The recoveries of authentic arachidonic acid and PGE_2 by ethyl acetate extraction were 87 and 95% respectively. Abbreviations: AA, arachidonic acid; PGE_2, prostaglandin E_2; and 6-$kF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$.

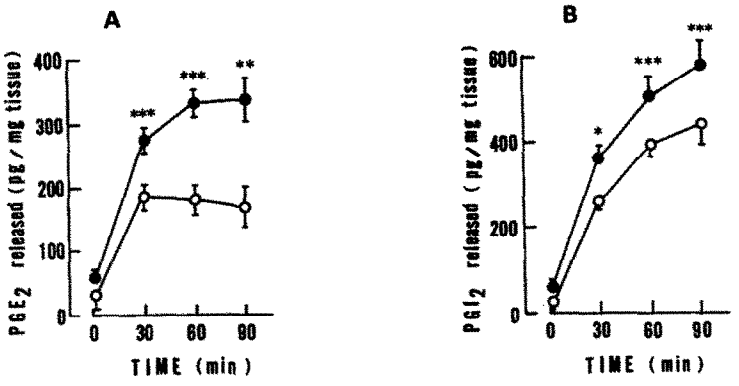

Fig. 3. Increase in PGE_2 and PGI_2 production in gastric-mucosa slices after incubation with the 1-COOH metabolite *in vitro*. Gastric-mucosa slices (approximately 200 mg) were incubated with the compound at 37° for the indicated periods in Krebs–Ringer solution (pH 7.4) containing 0.1% bovine serum albumin. The amounts of PGE_2 and PGI_2 (6-keto-$PGF_{1\alpha}$) released into the medium were radioimmunoassayed. Panels A and B show the PGE_2 and PGI_2 production respectively. Key: (○) controls; and (●) 100 μM 1-COOH metabolite. Each value represents the mean ± SEM of seven animals. (*) = $P < 0.05$, (**) = $P < 0.02$, and (***) = $P < 0.01$ when compared with the control.

aspirin-treated rats. Administration of aspirin (5 mg/kg) to rats decreased the production of PGI_2 from the gastric mucosa but the PGI_2 production was partially recovered by administration of plaunotol at 300 mg/kg (Fig. 5).

DISCUSSION

The present study demonstrates that plaunotol and its main metabolite, the 1-COOH metabolite, stimulated PGE_2 and PGI_2 production in the cultured cells of 3T6 fibroblasts. Plaunotol and its 1-COOH metabolite enhanced the release of arachidonic acid and the resultant PG production from [^{14}C]arachidonic acid prelabeled cells. This finding suggests that the stimulation of PG production by these compounds is ascribable to the activation of cellular phospholipase. The other plaunotol metabolites, which have two carboxyl groups in one molecule, and gefarnate, which has an isoprene structure similar to plaunotol, did not possess such stimulatory activity. Therefore, this stimulatory effect seems to be specific to plaunotol and its 1-COOH metabolite.

Hong *et al.* [28] reported that bradykinin and thrombin stimulate PGE_2 production several-fold in methylcholanthrene-transformed 3T3 fibroblast cells, and this increase was explained by activation of cellular phospholipase. Hassid and Levine [29] also reported that Melittin (bee venom peptide) activates phospholipase activity followed by an increase in PG production in fibroblast cells. It should be noted that plaunotol, a diterpene alcohol, is a stimulator of cellular phospholipase and promotes PG production in fibroblast cells.

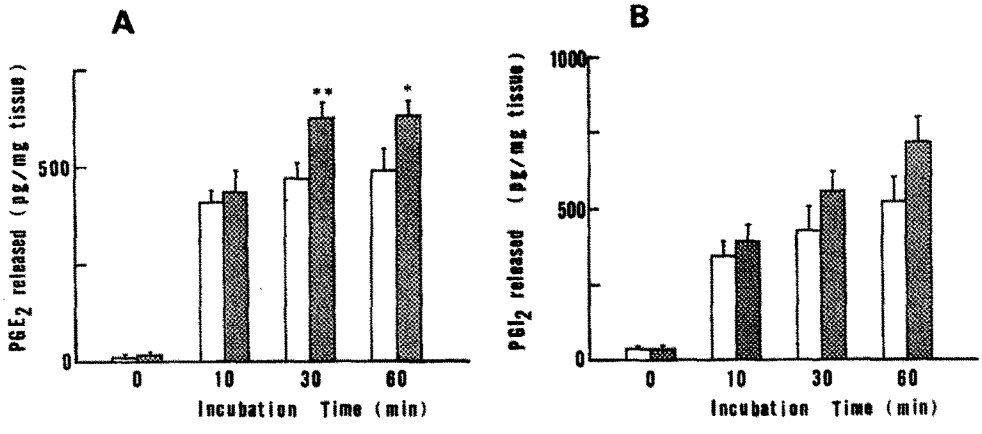

Fig. 4. Stimulation of gastric-mucosa PGE_2 and PGI_2 production in rats by oral administration of plaunotol. Plaunotol was administered orally to rats at a dose of 300 mg/kg. The rats were killed 90 min after the drug administration, and the stomach was taken out and the gastric mucosa was dissected into slices. The slices (approximately 300 mg) were placed in Krebs–Ringer solution and incubated at 37° for the indicated periods. The amounts of PGE_2 and PGI_2 (6-keto-$PGF_{1\alpha}$) released into the medium were determined as described in Materials and Methods. The control group received the vehicle only. Panels A and B show the PGE_2 and PGI_2 production respectively. Key: (□) control group; and (▨) plaunotol-administered group. Each value represents the mean ± SEM of nine animals. (*) = $P < 0.05$, and (**) = $P < 0.02$ when compared with the control group.

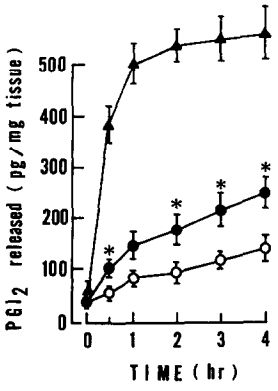

Fig. 5. Effect on gastric-mucosa PGI_2 production in aspirin-treated rats of oral administration of plaunotol. Plaunotol (300 mg/kg) or the vehicle was administered orally to rats 60 min before aspirin (5 mg/kg, p.o.) administration. The rats were killed 15 min after aspirin administration, the stomach was taken out, and the slices of the mucosa were prepared. The activity of PGI_2 production from the mucosa was determined as described in Materials and Methods. The control group received the vehicle only. Key: (▲) control group; (○) the vehicle and aspirin-administered group; and (●) plaunotol and aspirin-administered group. Each value represents the mean ± SEM of nine animals. (*) = $P < 0.05$ when compared with the vehicle and aspirin-administered group.

The stimulation of PG production in fibroblast cells was reproduced in rat gastric-mucosa slices. In rat gastric mucosa, plaunotol stimulated PGE_2 and PGI_2 production when the drug was administered orally to normal rats or aspirin-treated rats. The stimulation of PG production was clearly observed *in vitro* when the main metabolite of plaunotol was added to the incubation medium. In agreement with this finding, Sato *et al.* [30] reported that oral administration of plaunotol increases the PG content in gastric mucosa in cimetidine-treated rats. These results suggested that plaunotol could elevate the PG contents in the ulcer regions in the stomach. The elevated endogeneous PGs resulting from plaunotol administration may participate in its pharmacological activity, since the PGs are reported to increase local blood flow [12, 13], stimulate gastric mucus secretion [14] and have cytoprotective effects on gastric mucosa [15, 16].

Muramatsu *et al.* [31] reported that SU-88 (sofalcone) shows anti-cancer activity by inhibiting 15-hydroxy-PG-dehydrogenase (15-OH-PGDH) and elevating the PG level in gastric mucosa. Carbenoxolone (CBX), an anti-ulcer drug, was also reported to inhibit 15-OH-PGDH [32]. In our preliminary reports [33, 34], the 1-COOH metabolite of plaunotol also possessed inhibitory activity toward the 15-OH-PGDH partially purified from rat stomach. So, it seems possible that the increase of the PG level in the gastric mucosa by plaunotol may be due to the inhibition of PG degradation in addition to the stimulation of PG biosynthesis through the cellular phospholipase activation.

In the *in vitro* experiments using fibroblast cells and gastric-mucosa slices, plaunotol stimulated the PG production but its 1-COOH metabolite showed more potent stimulation. Komai *et al.* [35] reported that plaunotol is rapidly metabolized to the 1-carboxylic plaunotol (1-COOH metabolite) and only a trace amount of plaunotol is detected in plasma when the drug is administered orally to animals and humans. Thus, it might be speculated that plaunotol exerts its stimulatory activity of PG production after the conversion to the more active 1-COOH metabolite.

Acknowledgements—We are grateful to Drs. Shinsaku Kobayashi and Kiyokata Ohnishi of the Sankyo Co. for their valuable advice and comments, and to Miss Takako Kohriyama for her excellent technical assistance.

REFERENCES

1. A. Ogiso, E. Kitazawa, M. Kurabayashi, A. Sato, S. Takahashi, H. Noguchi, H. Kuwano, S. Kobayashi and M. Mishima, *Chem. pharm. Bull., Tokyo* **26**, 3117 (1978).
2. S. Kobayashi, C. Ishibashi, A. Morita, H. Masuda and A. Ogiso, *Pharmacometrics* **24**, 599 (1982).
3. S. Okabe, K. Tabata and H. Kunimi, *Pharmacometrics* **23**, 785 (1982).
4. A. Miyoshi, *Prog. Med.* **5** (Suppl. 1), 981 (1985).
5. K. Tsuneoka, N. Watanabe and T. Ohsawa, *Prog. Med.* **5** (Suppl. 1), 842 (1985).
6. K. Fukuda, K. Endo, M. Fukami, M. Makino and S. Kobayashi, *Prog. Med.* **5** (Suppl. 1), 813 (1985).
7. A. Robert, J. E. Nezamis and J. P. Phillips, *Am. J. dig. Dis.* **12**, 1073 (1967).
8. A. Robert, J. E. Nezamis and J. P. Phillips, *Gastroenterology* **55**, 481 (1968).
9. J. E. Nezamis, A. Robert and D. F. Stowe, *J. Physiol., Lond.* **218**, 369 (1971).
10. D. W. Wilson, C. Phillips and R. A. Levine, *Gastroenterology* **61**, 201 (1971).
11. M. Classen, H. Koch and J. Bickhardt, *Digestion* **4**, 333 (1971).
12. B. J. R. Whittle, N. K. Boughton-Smith, S. Moncada and J. R. Vane, *Prostaglandins* **15**, 955 (1978).
13. S. Moncada, R. J. Flower and J. R. Vane, in *The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), 6th Edn, p. 668. Macmillan, New York (1980).
14. J. P. Bolton, D. Palmer and M. M. Cohen, *Am. J. dig. Dis.* **23**, 359 (1978).
15. A. Robert, J. E. Nezamis, C. Lancaster and A. J. Hanchar, *Gastroenterology* **72**, 1121 (1977).
16. A. Robert, J. E. Nezamis, C. Lancaster and A. J. Hanchar, *Gastroenterology* **77**, 433 (1979).
17. A. Robert, J. R. Schultz, J. E. Nezamis and C. Lancaster, *Gastroenterology* **70**, 359 (1976).
18. A. Robert, in *Advances in Prostaglandin and Thromboxane Research* (Eds. B. Samuelsson and R. Paoletti), Vol. 2, p. 507. Raven Press, New York (1976).
19. S. M. M. Karim and W. P. Fung, in *Advances in Prostaglandin and Thromboxane Research* (Eds. B. Samuelsson and R. Paoletti), Vol. 2, p. 529. Raven Press, New York (1976).
20. K. Gibiński, J. Rybicka, E. Mikoś and A. Nowak, *Gut* **18**, 636 (1977).
21. K. Matsuda, K. Ohnishi, E. Misaka and M. Yamazaki, *Biochem. Pharmac.* **32**, 1347 (1983).
22. S. C. L. Hong and L. Levine, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1730 (1976).
23. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
24. H. Wagner and U. P. W. Horhammer, *Biochem. Z.* **334**, 175 (1961).
25. K. Matsuda, Y. Tanaka, S. Ushiyama, K. Ohnishi and M. Yamazaki, *Biochem. Pharmac.* **33**, 2473 (1984).

26. C. Takeguchi, E. Kohno and C. J. Sih, *Biochemistry* **10**, 2372 (1971).
27. B. Spławińska, J. Spławiński, T. Corell, G. Hasselmann, M. Nieszpaur and B. Jakubek, *Prostaglandins* **26**, 139 (1983).
28. S. C. L. Hong, R. P. Cynkin and L. Levine, *J. biol. Chem.* **251**, 776 (1976).
29. A. Hassid and L. Levine, *Res. Commun. Chem. Path. Pharmac.* **18**, 507 (1977).
30. H. Sato, T. Arakawa, T. Fukuda, T. Shigemoto, H. Sakuma, K. Higuchi, A. Nakamura, H. Nakamura, H. Yamada, S. Chono, T. Ono and K. Kobayashi, *J. adult Dis.* **15**, 525 (1985).
31. M. Muramatsu, M. Tanaka, T. Suwa, A. Fujita, S. Otomo and H. Aihara, *Biochem. Pharmac.* **33**, 2629 (1984).
32. B. M. Peskar, *Scand. J. Gastroent.* **15** (Suppl. 65), 109 (1980).
33. S. Ushiyama, K. Matsuda, F. Asai, T. Kohriyama, T. Oda, Y. Iijima, K. Ohnishi and M. Yamazaki, *Prog. Med.* **5** (Suppl. 1), 825 (1985).
34. M. Yamazaki, S. Ushiyama, K. Matsuda, F. Asai, Y. Iijima and K. Ohnishi, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. O. Hayaishi and S. Yamamoto), Vol. 15, p. 659. Raven Press, New York (1985).
35. T. Komai, T. Ikeda, K. Kawai, C. Handa and M. Tanaka, *Prog. Med.* **5** (Suppl. 1), 849 (1985).